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SHORT COMMUNICATION

Prevalence and distribution of human papillomavirus findings in swab specimens from gynaecology clinics of the east coast of Spain

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Abstract

The objective of this study was to determine the prevalence of human papillomavirus (HPV) among females in the east coast of Spain. A total of 1956 women visiting gynaecology clinics for routine check-ups were included in the study. Swabs were analyzed for HPV DNA by consensus polymerase chain reaction followed by direct sequencing. The overall HPV prevalence was 12.99%. HPV vaccine types 6, 11, 16 and 18 were detected in 6.13% of female participants.

Introduction

Cervical cancer is the second most common cancer among women worldwide [1]. Several epidemiological and laboratory-based studies have identified infection with any 1 of the defined high-risk (HR) human papillomavirus (HPV) types as a necessary, but not sufficient, cause of cervical cancer [2–4]. More than 100 HPV types have been detected and approximately half of them infect the genital tract [5]. We considered HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 as HR-HPV types, and HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89 as low-risk (LR) HPV types [6]; other HPV types were considered undetermined risk types (UR). HR-HPV types are detected in 99% of cervical cancers, and approximately 70% of cervical cancers worldwide are due to HPV types 16 and 18 [7–9].

In September 2006, the European Medicines Agency approved the use of the human papillomavirus vaccine (types 6, 11, 16, 18; recombinant, adsorbed; Gardasil®, Sanofi Pasteur MSD) for the prevention of cervical carcinoma (cervical cancer), high grade cervical dysplasia CIN2/3 (precancerous cervical lesions), high grade vulvar dysplastic lesions VIN2/3 (precancerous vulvar lesions) and external genital warts (condyloma acuminata) caused by human papillomavirus types 6, 11, 16 and 18 [10–13]. In Spain, this papillomavirus vaccine was included in the vaccination schedule of the National Health System in October 2007. Recommendations target a cohort of pre-adolescent girls aged 11–14 y [14]. In order to evaluate the efficacy of the vaccine and its effect on the rate and type distribution of HPV infection, it is necessary to study the prevalence and genotype distribution of HPV infection in a representative area of Spain for the period before HPV vaccine implementation. Hence, to determine a pre-vaccine population-based prevalence of cervicovaginal HPV, we performed HPV DNA testing on collected vaginal swabs from females visiting gynaecology clinics for routine check-ups in Valencia (Spain).

Materials and methods

Population and specimen collection

This study was coordinated by the Department of Pathology at the Consorcio Hospital General Universitario de Valencia, a Spanish National Health

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System institution. In order to obtain a representative population sample we considered 102 gynaecology clinics as the centres where samples (swabs) were collected to determine the presence of HPV DNA. All females aged 18–64 y, without previous knowledge of HPV infection, were eligible for participation in this study (January–September 2007). A total of 1956 females were considered in the study, all of whom live in Valencia (Spain). The commercial network of Sanofi Pasteur MSD was used to establish contact with these clinics. Written informed consent was obtained from all the participants.

HPV genotype determination

DNA isolation was performed by standard phenol–chloroform and proteinase-K protocols. The DNA of HPV was detected by nested polymerase chain reaction (PCR) using general consensus primers MY09/MY11 and GP5+/GP6. Appropriate DNA was valued with β-globin primers as an internal control for sample amplification. The second amplification cycle was performed using the LightCycler (Roche Diagnostics GmbH, Mannheim, Germany). PCR products were purified and directly sequenced with Cy5 labelled GP6+, using an ALFexpress II automated sequencer (GE Healthcare) [15,16]. For typing, the single nucleotide sequences obtained were aligned with the GenBank database [17], and the multiple superimposed sequences were used with the database of Feoli-Fonseca et al. [18].

Statistical analysis

To examine the association between age and overall HPV prevalence, age was categorized into 6 intervals: 18–24, 25–29, 30–34, 35–44, 45–54 and 55–64 y. For each HPV prevalence, the estimation of the ratio and 95% confidence interval (CI) was given. Statistical analysis was done using Pearson's Chi-square test. A p-value of <0.05 indicated statistical significance.

Results

The overall HPV prevalence was 12.99% (95% CI 11.50–14.48%) among females aged 18–64 y ($N=1956$). A single HPV was detected in 12.01% (95% CI 10.57–13.46%) and multiple HPV in 0.97% (95% CI 0.54–1.41%) of cases. HR-HPV prevalence was 9.30% (95% CI 8.02–10.59%), LR-HPV prevalence was 3.73% (95% CI 2.89–4.57%) and UR-HPV was 0.26% (95% CI 0.03–0.48%). The results grouped by age for the overall HPV prevalence, as well as for the single and multiple HPV prevalence, are shown in Table I.

HPV vaccine types 6 and 11 (LR types) and 16 and 18 (HR types) were detected in 6.13% (95% CI 5.07–7.20%) of female participants; HPV6 was detected in 0.72% (95% CI 0.34–1.41%), HPV11 in 0.97% (95% CI 0.54–1.41%), HPV16 in 3.94% (95% CI 3.07–4.80%), and HPV18 in 0.61% (95% CI 0.27–0.96%) of female participants. HPV prevalence of vaccine types 6, 11, 16, 18 and of the grouped vaccine types (HPV vac) are shown in Table II. The Chi-square for the HPV prevalence of vaccine types between categorized ages was significant ($p < 0.002$); the prevalence of the vaccine types was higher in women aged 18–34 y.

The overall prevalence of HR-HPV types was 9.39% and of LR-HPV types was 3.73%. HR-HPV prevalence was higher than LR-HPV prevalence ($p < 0.001$). Figure 1 shows the prevalence of HR- and LR-HPV types among categorized ages, where the prevalence of HR-HPV types was higher in women aged 30–34 y.

Chi-square for the HR-HPV prevalence was statistically significant for the age group 30–34 y vs 35–44 y ($p < 0.002$) and for the grouping 18–34 vs 35–64 y ($p < 0.001$). In comparison, Chi-square for the LR-HPV prevalence was statistically significant only for the age group 35–44 vs 45–54 y ($p < 0.05$).

We found 33 different HPV genotypes (in descending order according to prevalence): HPV16, HPV31, HPV11, HPV 54, HPV6, HPV81, HPV18,

Table I. HPV prevalence among categorized ages.

Age	n	HPV		Single HPV	Multiple HPV
		%	95% CI	%	%
18–24	295	15.6	11.5–19.7	14.5	1.2
25–29	437	13.7	10.5–17.0	12.2	1.6
30–34	396	17.3	13.6–21.0	16.5	0.9
35–44	582	8.0	5.8–10.2	8.0	0.0
45–54	192	8.8	4.8–12.8	8.8	0.0
55–64	54	3.7	0.0–8.8	3.7	0.0

HPV, human papillomavirus; CI, confidence interval.
Chi-square tests are: HPV prevalence among categorized ages: $p < 0.001$; 18–24 vs 25–29: non-significant; 25–29 vs 30–34: non-significant; 30–34 vs 35–44: $p < 0.001$; 35–44 vs 45–54: non-significant; 45–54 vs 55–64: non-significant; grouping 18–34 vs 35–64: $p < 0.001$.

Table II. HPV prevalence of vaccine types among categorized ages.

Age	HPV6	HPV11	HPV16	HPV18	HPV vac	95% CI
18–24	1.2%	0.0%	5.2%	1.2%	7.5%	4.5–10.5%
25–29	0.8%	3.5%	3.9%	1.2%	9.4%	6.7–12.2%
30–34	0.0%	1.7%	6.9%	0.0%	7.8%	5.2–10.4%
35–44	0.0%	0.0%	2.7%	0.0%	2.7%	1.4–4.0%
45–54	3.5%	0.0%	0.0%	0.0%	3.5%	0.0–8.4%
55–64	0.0%	0.0%	0.0%	0.0%	0.0%	0.0–0.0%

HPV, human papillomavirus; CI, confidence interval.
Chi-square tests for HPV vac are (similar results to Table I): HPV vac prevalence among categorized ages: $p < 0.001$; 18–24 vs 25–29: non-significant; 25–29 vs 30–34: non-significant; 30–34 vs 35–44: $p < 0.001$; 35–44 vs 45–54: non-significant; 45–54 vs 55–64: non-significant; grouping 18–34 vs 35–64: $p < 0.001$.

HPV58, HPV66, HPV56, HPV52, HPV53, HPV33, HPV45, HPV59, HPV62, HPV90, HPV39, HPV72, HPV73, HPV30, HPV42, HPV70, HPV91, HPV35, HPV55, HPV67, HPV68, HPV82, HPV83, HPV84, HPV89, HPV102. Table III shows the prevalence of HPV types with $n \geq 5$.

Discussion

The prevalence of HPV DNA in a representative sample of Spanish women aged 18–64 y was 12.99%, with the highest prevalence (17.30%) in women between the ages of 30 and 34 y. The Spanish census of 2007 [19] counted approximately 14.5 million women in Spain, and 1.5 million in the Valencian community for the age groups included in the present study. This total prevalence may correspond to approximately 1.8 million women between 18 and 64 y of age with HPV infection in Spain and 190,000 women in the Valencian community.

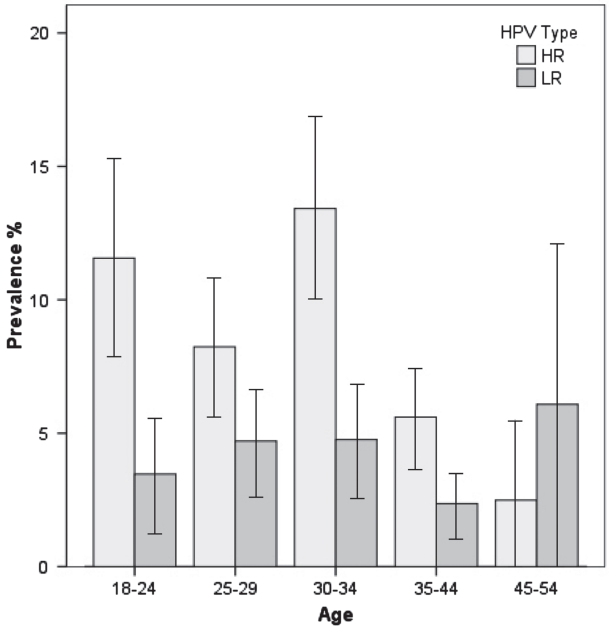

Figure 1. Prevalence of HR (high-risk) and LR (low-risk) HPV types among categorized ages. Error bars indicate 95% confidence intervals.

The values of overall HPV prevalence in eastern Spain determined in the present study (12.99%) are higher than those from the study of De Sanjose et al. [9] carried out between 1998 and 2000 (overall prevalence of 3%), but our HR-HPV prevalence results are similar to those of Ortiz et al. [20] in the overall population in 2004 (9.39% in our study vs 10.7% in Ortiz et al.). Although all these studies have used similar technology and methods for HPV DNA detection (L1 consensus PCR), differences in the population analysis may exist. It is risky to confirm an increment in the prevalence of HPV infection, although a tendency of higher results is evident.

In this study, the HR-HPV prevalence was 9.39% of all female participants, where HPV16 was detected in 3.94% and HPV18 in 0.61%, which corresponds to 41.95% and 6.5% of detected HR-HPV, respectively. The HR-HPV prevalence determined in the largest studies from the European Union varies from 2.5% in Greece [21] to 22.8% in Denmark [22]. In Italy, the prevalence was found to be 9.4% in 2005

Table III. Prevalence of HPV types.

HPV type	*n*	%	95% CI
16	77	3.94	3.07–4.80%
31	27	1.38	0.86–1.90%
11	19	0.97	0.54–1.41%
54	15	0.77	0.38–1.15%
6	14	0.72	0.34–1.09%
81	13	0.66	0.30–1.02%
18	12	0.61	0.27–0.96%
58	10	0.51	0.20–0.83%
66	10	0.51	0.20–0.83%
56	9	0.46	0.16–0.76%
52	8	0.41	0.13–0.69%
53	7	0.36	0.09–0.62%
33	6	0.31	0.06–0.55%
45	6	0.31	0.06–0.55%
59	5	0.26	0.03–0.48%
62	5	0.26	0.03–0.48%

HPV, human papillomavirus; CI, confidence interval.

[23], and in France it was 14.3% in 2001 for HR-HPV [24]; in the UK it has varied from 11.2% to 15.7%; in Ireland 19.8%, in Finland 7.5%, in Belgium 15.2%, etc. In these studies, the HPV16 prevalence among HR-HPV varied from 18.7% to 41.1%, and the HPV18 prevalence among HR-HPV varied from 6.4% to 21.7% [25].

By comparing the prevalence data from our study with studies in other world countries, the prevalence in the Asian countries (studies performed between 2003 and 2006) is similar to ours in most cases [26–30]. South American countries also present similar prevalence to ours (studies performed between 2001 and 2004) [31–33]. The prevalence reported in some African countries is about 30% [34,35], and the last study from the USA, performed by the Centers for Disease Control and Prevention, records 26.8% [36].

The prevalence of multiple HPV was found to be 0.97% of all patients (8.1% of all positive samples). This prevalence is lower than those reported in the studies that use hybridization for the detection of final amplifications [37]. One of the advantages of direct sequencing used by us in relation to hybridization technology is the absence of limits in the search of HPV genotypes. In fact, the hybridization technology has a higher sensitivity in the detection of multiple infections (sensitivity of identifying minority variants). In direct sequencing, amplifications with multiple sequences in percentages lower than 20% may not be detected when a majority sequence exists [37].

In our opinion, the genotype distribution in the different studies published is biased by the detection method used: the Sanger dideoxy sequencing method used in this study is restricted to the detection of the most amplified fragment among all the amplified HPV types, without considering minor variant sequences. The methods that use hybridization detect minor amplified sequences but are dependent on the detection probes used.

It is probable that if we had used the pyrosequencing technology as a detection method (analytical sensibility near to 1%, which does not have the interpretation problem seen in the Sanger method, and without the problems of the hybridization restriction), we could have discovered that most of the samples without cervical lesions show several types of HPV [38,39].

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